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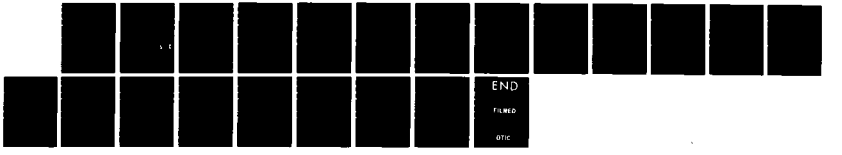

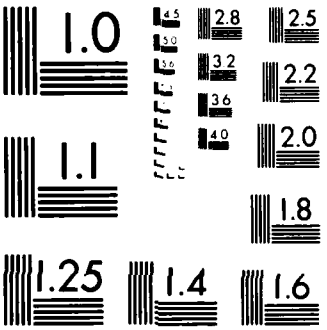

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LASSA FEVER IMMUNE PLASMA

Annual Summary Report

John D. Frame, M.D.

August 1983

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD17-79-C-9024

Trustees of Columbia University
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New York, N.Y. 10032

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Collection of Lassa Fever Immune Plasma Units has continued with collection of 79 units of the 100 projected for 1983. 52 units have been forwarded to USAMRIID. Identification of Lassa fever (LF) patients has continued at Curran Lutheran (CLH) and Phebe (PH) Hospitals, and to some extent in other hospitals as well. 15 LF cases and 9 presumptive cases were diagnosed at CLH; of 88 patients in whom virus isolation was attempted, virus was isolated in 10. At PH, six of 52 consecutive febrile patients had LF; virus isolation

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has not been performed as yet in these and other patients from whom serum has been obtained.

Village surveys in Kolahun District showed prevalences of Lassa virus antibodies of 6.2% and 8.6%, at screening titers of 1:8.

An ultra freezer has been purchased for CLH to permit more adequate determination of the degree of viremia as the basis for future investigations of the clinical efficacy of LFIP. Plans are under way to obtain a refrigerated centrifuge and ultra-freezer for PH so that it can become a second field station for plasmapheresis and for clinical investigations.

Summary

The collection of Lassa Fever Immune Plasma continued in Liberia with plasmapheresis at the Curran Lutheran Hospital (CLH) in Zorzor. Seventy-nine LFIP units of a planned 100 units have been obtained through June, and 52 forwarded to USAMRIID; it is expected that by the end of 1983 more than 100 units will have been collected.

As a means of identifying potential plasma donors and in order to continue the collection of information regarding the importance of Lassa fever (LF) in Liberia, surveys of febrile patients have continued. At CLH 15 cases of LF were diagnosed by serological and virological techniques among 146 patients tested from November 1982 through April 1983; of these, 10 were identified by means of virus isolation among the first 88 patients. Virus isolation has not yet been attempted in the last 58 patients. Six cases of LF were diagnosed serologically among 52 patients tested consecutively in Phebe Hospital (PH) in November 1982. Testing of patients continues at CLH, PH and a number of other Liberian hospitals.

Two village surveys demonstrated prevalences of 6.2% and 8.6% in Mbabahun and Korworhun, two communities in Kolahun District where further studies are contemplated as a means of elucidating the epidemiology of LF.

Ultra-freezers have been purchased for CLH to enable more accurate determinations of viremias in patients there. Plans are underway to add PH as a center for plasmapheresis, and to obtain a refrigerated centrifuge and ultra-freezer for that institution.

Mr. J. E. Yalley-Ogunro, Field Investigator and Resident Head, and Andrew Cole, M.D., Clinical Investigator, both Liberians, conduct the work in Liberia, under the general direction of John D. Frame, M.D., Principal Investigator. The latter traveled to Liberia in January and expects to return in October.

FORWARD

For the protection of human subjects the investigator(s) have adhered to polocies of applicable Federal Law 45CFR46.

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I. Statement of the Problem

Lassa fever (LF) was first found as a disease of high morbidity and mortality in nosocomial outbreaks in West Africa (1,2,3). Subsequently it was learned that Lassa virus (LV) infections are widely spread throughout the region (4). Treatment of cases is essentially supportive; specific treatment with LF Immune Plasma (LFIP) has been used with equivocal results (5). Other rational therapeutic measures await better knowledge of the pathogenesis and pathology of human LV infections.

Prevention of LF requires elucidation of its epidemiology, and will likely require the preparation and use of a vaccine. However, investigations into LV infection carry a definite risk (6), and measures should be available to protect investigators as well as patients.

Thus, research into the epidemiology and pathogenesis of LF, in the nature of the virus, and in the development of a preventive vaccine appear to be of high priority if this hemorrhagic fever is to be brought under control.

II. Background

An outbreak of LF in the Curran Lutheran Hospital (CLH), in Zorzor in 1972 demonstrated its presence in Liberia (5). A pilot study conducted from 1976 to 1979 revealed high prevalences of LV antibodies (LVA) in members of hospital staffs in Liberia, and the feasibility of working with Liberian hospitals in further investigations of LF in that country (7). It also persuaded the Republic of Liberia to agree to ongoing research in LF. On the basis of the preliminary findings, a joint program to procure LFIP and to study the epidemiology of LF was entered upon by Columbia University (CU), the Liberian Institute for Biomedical Research (LIBR) and the United States Army Medical Research Institute of Infectious Diseases (USAMRIID) under Contract DAMD17-79-C-9024 awarded by U.S. Army Medical Research and Development Command (USAMRDC).

Initial efforts to obtain LFIP of adequate protective capacity against LF were limited by the absence of definite criteria for the selection of donors. During the course of initial plasma collections it was found that donors did not develop adequate neutralization titers against LV until 3 to 9 months after the onset of the disease (8). In addition it was discovered that in some donors a "cycling" phenomenon occurred so that a donor with adequate neutralizing antibodies (NA) as determined by the Log Neutralization Index (LNI) might well have very low titers several weeks later, only to demonstrate good titers subsequently (9). Furthermore, the discovery of several strains of LV demonstrated that a person who had been infected by one strain might not have an adequate LNI against another type of LV (9).

Eventually of 122 units of LFIP obtained, 75 were found to meet criteria satisfactory to USAMRIID: 58 of 97 LFIP units submitted to USAMRIID met the criteria. The other 17 "good" units were left in Liberia for treatment of patients there.

In the meantime, investigations among hospitalized patients in northern Liberia revealed LF to be the cause of illness in 10 to 15% of febrile patients, in some cases, at least, being the most common cause of fever among adults in the hospital. Further investigations among hospital personnel showed prevalences of LVA varying from 4 to 40%, and indicated that LV infections are endemic in all regions of Liberia. Village surveys, for the most part in communities near CLH and the Swedish Free Pentecostal Mission Clinic (SMC) in Foya Kamara, near the Sierra Leone border, revealed LVA prevalences of 0.9 to 14.1%. LV was isolated 38 times from 31 patients, not only at CLH,where attempts at isolation first made, but also in Phebe Hospital (PH), Bong County, 80 miles southeast of Zorzor.

III. Approach to the Problem

Plasma donors were identified by continued serological testing of febrile patients in hospitals, and by attempts at virus isolation. Potential donors were requested to submit sera for testing for LV NA, and those with adequate LNI were asked to submit to plasmapheresis.

Epidemiological investigations were carried out among patients found to have LF, and by means of village surveys for the prevalence of LF; villages were selected for differences that might elucidate factors contributing to varying prevalences of LF.

IV. General Narrative

During the early part of the year the Field Investigator, Mr. J. E. Yalley-Ogunro, traveled repeatedly from his base at the LIBR near Robertsfield to CLH to conduct plasmapheresis, and to collect sera obtained from febrile patients at CLH, PH and other hospitals and clinics in the North. Upon his return to his laboratory at the LIBR he tested specimens for IFA by means of the fluorescence microsocpe there, using antigen spot slides supplied by USAMRIID.

In February the Principal Investigator, John D. Frame, M.D., traveled to Liberia. He visited Ph and CLH to review the collection of patient sera, and to discuss further work with the medical staff there. He then traveled with Mr. Yalley-Ogunro and Andrew Cole, M.D., the Clinical Investigator, to Kolahun, 100 miles to the West, to investigate the possibilities of further work in that area. It is in this district that the SMC is located; clinic personnel have the highest prevalence of LVA found in health workers in Liberia, and it was believed that work in the district might lead to the discovery of many plasma donors, possibly with antibodies to types of LV not seen frequently in CLH. Preliminary plans were made for future surveys in villages and Health Center Posts in the region.

Upon return to CLH the Principal Investigator and Field Investigator conducted further plasmapheresis, and the former returned to the United States with 36 units of plasma for USAMRIID. In April Mr. Yalley-Ogunro and Dr. Cole conducted village surveys in Kolahun District. Subsequently Dr. Cole continued to work with health personnel at the SMC and the Tellewoyan Government

Hospital, between the CLH and Kolahun, to systematize the collection of sera from febrile patients in these institutions.

Upon his return to the United States Dr. Frame took steps to obtain ultra-freezers to permit the storage of specimens at CLH and the LIBR at such low temperatures that the virus isolation and the determination of virus titers could be made with confidence. This has been requested earlier by Dr. Jahrling so that true values for the titers of viremia could be determined at CLH, and some understanding gained of the severity of infections being treated in that hospital.

Mr. Yalley-Ogunro made further trips to CLH for plasmapheresis in April and June, and at his home base continued testing sera for LVA.

During a conference between Dr. Frame and Dr. Peter B. Jahrling of USAMRIID in April, Dr. Jahrling noted that even under rather unfavorable circumstances surrounding the work at PH he was isolating LV from patients in that institution. He suggested it would be valuable to attempt more systematic investigations there. After correspondence with Dr. Walter Gwenigale, Medical Director at PH, and Dr. John Fredell, Chief of Medical Services, their willingness to collaborate in work at their hospital was obtained, and steps started to obtain a refrigerated centrifuge and appropriate freezer to make this possible. When the ultra-cold freezer and centrifuge are in place at PH, plasmapheresis will be possible there.

V. Results

A. Plasmapheresis

Seventy-nine LFIP units have been obtained to date, toward the goal of 100 units for this year. Fifty-two have been delivered to USAMRIID; and the remainder are to be sent when reliable means of transport can be obtained. In former years one shipment was delayed en route, and it is imperative that such a lapse not be repeated.

The units collected, and the donors, are listed in Appendix A. At the time of this report up-to-date testing for LNI had not been completed by Dr. Jahrling, though all donors had shown adequate titers on earlier testing.

B. Lassa fever patients

Diagnostic testing of febrile patients for the presence of LF continued in a number of hospitals (Appendix B). The discovery of LF cases serves to increase knowledge of the clinical disease, and to educate Liberian health workers in its diagnosis and management. It is also the prime means for the identification of potential donors of LFIP.

Diagnosis involves both serological testing at the LIBR, using the fluorescence microscope there, and attempts at isolation of virus from patients, performed at USAMRIID. The latter measure is only justified if it is possible to maintain specimens frozen to maintain active LV.

As in the past, most consistent testing was performed at the CLH in Zorzor. At times the pressure of other hospital duties in an understaffed hospital interfered with the complete investigation of potential LF cases. However, 88 patients with fever were tested between November, 1982 and February, 1983. Among them there were 12 cases of LF confirmed by virus isolation or seroconversion, and 5 other presumptive cases defined as patients with an IFA titer of 1:64 or higher, where two appropriately spaced sera were not available. Seven of the 12 proved cases were found by virus isolation only, only five cases would have been proved by seroconversion; another would have been considered presumptive on the basis of serological tests. Subsequently another 58 patients were tested serologically in March and April; virus isolation has not been attempted on these patients as yet. The importance of virus isolation as a means of early diagnosis is demonstrated by the considerably lower incidence of LF found in this group of patients.

In November, 1982 Dr. Andrew Cole conducted a survey of all patients with fever admitted to PH. Four cases of LF and 2 cases of presumptive LF were found by serodiagnosis among 52 admitted. Virus isolation has not been attempted on this group of patients as yet.

Dr. Cole attempted as well to encourage testing for LF in the [illegible] W. Harley Memorial Hospital in Ganta; there is no permanent physician [illegible] present. A physician supervising the adjacent rehabilitation cente[illegible] for leprosy patients asked him to test three patients with fever in her care; two were presumptive cases of LF with high IFA antibodies, the third may have ha[illegible], but in the absence of paired sera the diagnosis could not be made.

Dr. Cole also encouraged testing for LF in the SMC at Foya Kamara, and in the Tellowoyan Government Hospital at Voinjama. No proved cases of LF were identified in either institution. He is continuing efforts to encourage the collection of serum pairs from patients with fever in these centers when general population and hospital staff surveys have indicated a high prevalence of LF. Virus isolation attempts in these places will be wasted at this time; none has a reliable freezer.

Since the beginning of the Lassa Fever Immune Plasma program in Liberia it has seemed clear that the testing for IgM antibodies would be appropriate as a means of permitting the distinction between active LF, and the presence of residual antibodies from previous disease. Several previous attempts to evaluate the use of anti-human-IgM were frustrated by various misadventures. Finally, studies were carried out to compare the results of tests for IgM with our previous tests which used FITC against all human immune globulins. The results of the tests were disappointing. IgM could not be detected by this system in most patients who seroconverted, and who have subsequently been confirmed as cases of LF. In no instance did the presence of IgM confirm the presence of LF in a patient whose clinical history strongly suggested the diagnosis, and in whom previous serological tests had been negative or equivocal. The results of some of the tests are given in Appendix C.

Our failure to detect IgM in this system was paralleled by the experience of Dr. Peter Jahrling of USAMRIID. However, he is now attempting to develop an Enzyme-linked immunosorbent assay for IgM. Perfection of such a system would

make possible the diagnosis of LF under field conditions and in the hospitals where cases occur.

C. Village surveys

Following up previous investigations of the prevalences of antibodies to LV in selected Liberian villages, it was decided to survey four villages in Kolahun District where the prevalence of LF appears to be high. Villages were selected comprised of people in a single language group, the Bandi. All four were adjacent to the main highway passing through the District. Because of our previous suggestive finding, that LV infections were less common in villages that maintained traditional village cleanliness, with clean-swept areas between houses, two communities were selected that were "traditional", and two in which the areas between dwellings appeared to be unkempt. One pair of villages has been surveyed this year to date.

Mbabahun and Korworhun were the two villages surveyed. The former is clean-swept, the latter shows a lack of traditional sanitary discipline. Mbabahun is somewhat the larger. Dr. Cole and Mr. Yalley-Ogunro conducted the surveys by techniques previously described. After agreement by the village chief and elders, and with the consent of the villagers, the inhabitants were requested to present themselves on the selected date. They were registered by households, and tested by means of finger-tip blood obtained with the use of sterile disposable lancets. The specimens were collected on filter paper discs, if two discs are used for each subject they have been found to hold at saturation about 0.6 ml. blood. The specimens were dried, stored in a freezer, and taken to the LIBR to be tested for the presence of IFA. It has been found that after appropriate dilution the screening titer is approximately 1:8.

Tests were conducted on 370 inhabitants of Mbabahun, of which 15 were positive at the screening titer, and 8 gave equivocal results, for prevalences of 4.1% and 6.2%, if equivocal results were included. In Korworhun with 230 inhabitants tested there, 13 positive and an additional 7 with equivocal results, for prevalences of 5.7 and 8.6% respectively. The differences in the prevalences of LV antibodies was not significant. However, not all titrations have been carried out to end-points. It has been found in previous surveys that differences in the pattern of titers between populations gives an indication of varying epidemiological patterns in them.

VI. Conclusion

During this year the continued presence of LF in Liberia has been demonstrated. With the equipment obtained for CLH, and possibly for PH as well, field investigations of the use of LFIP in the treatment of patients will now be possible, with adequate monitoring of viremia at USAMRIID.

Further investigations of the incidence and epidemiology of LF in Liberia will be continued in the coming year under the guidance of the Clinical Investigator who is residing at the center of Kolahun District, a highly endemic region for LF in Lofa County.

With the new field station at PH it is expected that 50 units of LFIP will

be obtained in the coming year in addition to the 100 from plasmapheresis at CLH. By the second year at PH the total from the two field stations should be approximately 200 LFIP units per annum. This amount should ensure adequate supplies for investigations at USAMRIID, and for investigations into the therapy of LF in Liberia.

VII. References

1. Frame, J.D., Baldwin, J.M., Jr., Gocke, D.J., & Troup, J.M. Lassa fever, a new virus disease of man from West Africa. Clinical description and pathological findings. Am. J. Trop. Med. Hyg. 19: 670-675 (1970).

2. White, H.A. Lassa fever. A study of 23 hospital cases. Trans. Roy. Soc. Trop. Med. Hyg. 23: 1131-1139 (1972).

3. Monath, T.P., Mertens, P.E., Patton, R., Moser, C.R., Baum, J.J., Pinneo, L., Gary, G.W., & Kissling, R. A hospital outbreak of Lassa fever in Zorzor, Liberia, March-April, 1972. Amer. J. Trop. Med. Hyg. 22: 773-784 (1973).

4. Frame, J.D. Surveillance of Lassa fever in missionaries stationed in West Africa. Bull. W.H.O. 52: 593-598 (1975).

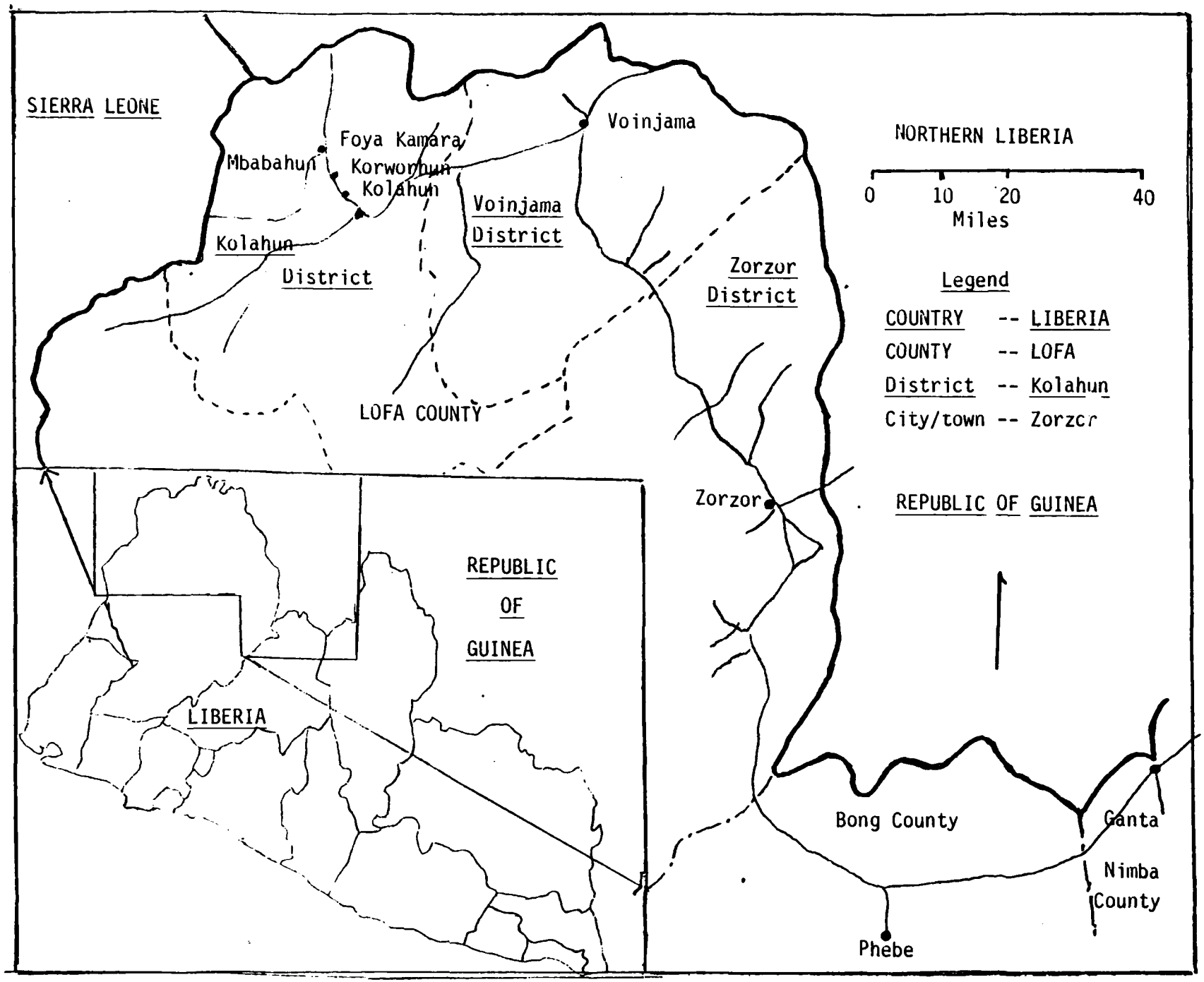
5. Clayton, A.J. Lassa immune serum. Bull. W.H.O. 55: 435-439 (1977).

6. Leifer, E., Gocke, D.J., & Bourne, H. Lassa fever, a new virus disease of man from West Africa. II. Report of a laboratory acquired infection treated with plasma from a person recently recovered from the disease. Am. J. Trop. Med. Hyg. 19: 677-679 (1970).

7. Frame, J.D., Casals, J. & Dennis, E.A. Lassa virus antibodies in hospital personnel in Western Liberia. Trans. Roy. Soc. Trop. Med. Hyg. 73: 219-224 (1979).

8. Jahrling, P.B. Arenaviruses. In: Manual of Clinical Microbiology, Lennette, E.H., Hansler, W.J., Jr., and Truant, J.P., Editors, 3rd edit. Washington, D.C.: American Society for Microbiology, pp. 884-890, 1980.

9. Frame, J.D. Annual Summary Report No. 3, Lassa fever immune plasma, Division of Tropical Medicine, School of Public Health, College of Physicians and Surgeons, Columbia University, New York, June 1983.



MAP -- NORTHERN LIBERIA

Appendix A. Lassa Fever Immune Plasma Units Collected in Liberia December 1982 - June 1983.

Name of Donor	Date of Illness	IFA Date	IFA Titer*	Neutralization Date	Neutralization LNI: Josiah	Neutralization Macenta	Date of Plasmapheresis	No. Units
YaK	10/81	12/09/81	512	01/12/82	1.2	1.5	12/82	2
		01/12/82	512	05/05/82	2.3	3.2+	01/83	2
		01/23/83	16					
DaK	10/81	12/09/81	512	01/12/82	1.2	1.5	12/82	2
		01/12/82	512	05/05/82	2.3	3.2+	02/83	2
		10/01/82	64	08/13/82	3.1+	3.0+	06/83	2
		02/21/83	128	10/09/82	3.1+	3.0+		
				02/21/83	2.4+	2.7+		
DaD	04/77	02/23/83	4	05/01/81	1.2	0.6	12/82	2
				12/14/82	0.2	--	02/83	2
				02/23/83	0.9	0.9	06/83	2
GaV	01/82	01/21/82	512	08/11/82	1.1	1.6	12/82	2
		01/23/83	32	11/02/82	2.2	2.5	02/83	2
				12/14/82	2.4+	2.4+		
JaM	?1974	12/01/81	16	01/13/82	0.0	0.0	12/82	2
		01/13/82	16	05/08/82	0.2	0.6	02/82	2
		12/15/82	8	08/12/82	0.0	0.0	06/83	2
		02/23/83	8	10/09/82	1.1	0.1		
YaV	01/82	01/21/82	1048	11/02/82	1.3	1.7	12/82	2
		02/23/83	64	02/23/83	2.4+	2.7+	02/83	2
MaZ	07/82	08/09/82	128	12/16/82	0.3	--	12/82	2
		02/25/83	128	02/22/83	0.9	0.7	02/83	2
							06/83	2
KoD	05/77	02/25/83	8	05/02/82	1.5	1.3		
				12/16/82	0.3	0.8	12/82	2
				02/23/82	2.4+	2.7+	02/83	2
BoK	10/82	11/02/82	8	12/16/82	0.2	0.1	12/82	2
		02/23/83	1	02/23/83	2.4+	2.7+	06/83	2
							06/83	2

Appendix A. (Con't.) Lassa Fever Immune Plasma Units Collected in Liberia December 1982 - June 1983.

Name of Donor	Date of Illness	IFA		Neutralization			Date of Plasmapheresis	No. Units
		Date	Titer*	Date	LNI: Josiah	Macenta		
LoF	01/82	01/08/82	1024	10/09/82	0.2	0.4	12/82	2
							04/83	2
DoM	05/81	01/12/82	256	01/12/82	0.0	0.8	12/82	1
		12/17/82	8	05/12/82	0.4	0.8		
				12/17/82	2.4+	2.4+		
MuS	11/81	11/25/82	1024	10/09/82	3.1+	3.0+	12/82	2
		04/23/83	64				04/83	2
KeKo	10/81?	02/21/83	64	02/21/83	2.4	1.5	02/83	2
DaB	11/82	12/23/82	128	02/21/83	2.4+	2.7+	02/83	2
							06/83	2
KeKa	10/82	12/17/82	8	12/17/82	2.4+	2.7+	02/83	2
KoM	?	04/23/83	20	04/23/83	0.5	0.5	04/83	2
KlM	12/80	04/23/83	64	05/01/81	2.2	2.4	04/83	2
HuT	1981	10/04/82	32	10/04/82	0.3	0.4	04/83	2
AnS	09/82	09/15/82	256	04/26/83	3.4+	3.1+	04/83	2
BeV	?	02/24/83	4	02/24/83	2.4+	1.5	06/83	2
DaZ	10/79	10/30/79	32	01/81	0.2	0.2	06/83	2
YaG	10/82	06/16/83	±	06/26/83	0.2	0.2	06/83	2
Total								79

* IFA titers expressed by the reciprocal

Appendix B. Surveys of Patients Hospitalized for Fever for the Presence of Lassa Virus Antibodies (IFA).

The testing of febrile patients admitted to hospitals has continued and the results of the tests are shown in the Table.

Criteria for the diagnosis of Lassa fever by serological and virological testing are as follows:

Lassa fever, confirmed by:

a. Virus isolation at USAMRIID
b. Seroconversion or four-fold rise in IFA titers

Presumptive Lassa fever, determined by the presence of Lassa virus antibodies at an IFA titer of at least 1:64.

Other positives, indicate patients whose sera LVA were present at titers of 1:32 or lower.

To date, attempts at virus isolation during this report year have been made only at Curran hospital through the end of February, 1983. No specimens have been forwarded to USAMRIID since early March.

Comparison of the results at Curran Lutheran Hospital during the period November 1982 through February 1983, with the other series indicates that the lack of virus isolation is accompanied by a much lower incidence of confirmed Lassa fever. Three of the ten LF patients diagnosed by virus isolation were also diagnosed by sero-conversion; the other 7 would have been missed without recovery of the virus, either because of death, or because of inadequate serum pairs. It may be assumed that many LF patients are missed in the other patient series.

Attempted isolation of virus is not worthwhile at Ganta, Foya and Voinjama; refrigeration is not adequate for the likely preservation of virus.

At Ganta three of the patients tested were from the leprosy rehabilitation project. All three were positive for LVA, and in two the diagnosis of LF could be made on presumptive grounds.

Appendix B (Con't.) Surveys of Patients Hospitalized for Fever for the Presence of Lassa Virus Antibodies (IFA).

Hospital (Dates)	No. Tested	Lassa fever				Presumptive Lassa fever (titers)	Total		Others positive
		Virus Isolation	Seroconversion or rise in IFA	Total No.	%		No.	%	
Curran (11/82-2/83)	88	10	2	12	13.6	5	17	19.3	9
Curran (3/83-4/83)	58	*	3	3	5.2	4	7	12.1	7
Phebe (11/82)	52	*	4	4	7.7	2	6	11.5	2
Phebe# (12/82-2/82)	52	*				1	1	2	4
Ganta#	5	*				2	2	40	1
Foya#	7	*							1
Voinjama	12	*							2

* Virus isolations not done.

\# Serum pairs tested in only a minority of patients.

Appendix C. The Diagnosis of Lassa Fever in Febrile Patients: Comparison of IgM and IgG titers.

The serological diagnosis of Lassa fever has been made on the basis of rising titers of IFA antibodies using a goat-anti-human-globulins conjugate. Presumptive Lassa fever has been diagnosed if the IFA titer was 1:64 or higher in instances when a appropriate serum pairs were not available for testing. It has seemed from the beginning of the program in Liberia that testing for IgM antibodies would be a rational means of diagnosing active LF, for these antibodies would be expected to disappear after active disease.

Tests for IgM class antibodies were carried out on 85 specimens in which the clinical history or seroconversion in tests performed earlier suggested that the patient was an active case of LF. Cappel goat-anti-human-IgM FITC was used at a dilution of 1:50. In all, 115 tests were performed. In no case did the presence of IgM clarify the diagnosis. The following specimens are listed as instances in which LF was confirmed by seroconversion or high IFA titers using goat-anti-human-globulin FITC; they represent the lack of sensitivity of testing with anti-human-IgM conjugate.

Patient No.	Anti-human-globulins FITC	Anti-human-IgM FITC
Z-171	Seroconversion to 1:64	neg
Z-201	" " 1:512	neg
Z-204	" " 1:64	neg
Z-243	Clinical history, titer 1:64	1:8
Z-248	Seroconversion to 1:8	neg
Z-257	" " 1:8	neg
Z-333	" " 1:32	neg
Z-363	Clinical history, titer 1:512	?
Z-546	Seroconversion to 1:256	1:32

Tests of the same type have also been performed by Dr. Peter Jahrling of USAMRIID (Personal communication), with comparable results. At present Dr. Jahrling is investigating another technique, that of Enzyme Linked Immunosorbent Assay, as a means of detecting IgM, and thus making early diagnosis of LF possible in the field. His initial results have been promising (Personal communication).

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